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Effects of dietary superoxide dismutase on growth performance, antioxidant capacity and digestive enzyme activity of yellow-feather broilers during the early breeding period (1–28d)

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ABSTRACT. The purpose of this study was to explore whether the addition of feeding superoxide dismutase (SOD) to yellow feather broilers in the early stages of growth could affect their growth performance, antioxidant properties, digestive enzyme activity and blood biochemical indices. A total of 320 male one-day-old yellow-feather broilers were evenly divided into 5 groups of 8 replicates each, with 8 chickens in each replicate. The control group was fed the basal diet, and four experimental groups were fed the basal diet + 100 mg/kg, 200 mg/kg, 400 mg/kg, and 800 mg/kg SOD, respectively. SOD was provided in powder form and the enzyme activity was 20 000 IU/g. Pre-feeding the basal diet for three days was followed by feeding the experimental diet for the trial period of 28 days. The results indicated that SOD significantly increased final body weight and average daily gain ($P < 0.05$) and significantly decreased the feed:gain ratio ($P < 0.05$) of broilers without affecting average daily feed intake ($P > 0.05$). Dietary SOD increased total antioxidant capacity and glutathione peroxidase activity (GSH-Px) ($P < 0.05$), reduced serum malondialdehyde ($P < 0.05$) levels, and increased total SOD, catalase and GSH-Px ($P < 0.05$) activities in the liver. The results demonstrated that dietary SOD supplementation improved the antioxidant capacity of broilers. However, SOD did not affect the activity of digestive enzymes (α-amylase, trypsin, lipase) in the duodenum, jejunum and ileum ($P > 0.05$). In addition, SOD reduced serum alanine transaminase and aspartate transaminase ($P < 0.05$) activity, indicating reduced burden on the liver. Based on these results, it can be concluded that the addition of different SOD concentrations to the diet can enhance the growth performance and antioxidant capacity of broilers. The addition of SOD provided the optimum effect in the range of 400–800 mg/kg.

Introduction

Free radicals are continuously produced during cellular respiration and normal metabolism (Di Meo and Venditti, 2020). These highly reactive molecules, such as reactive oxygen species (ROS), are closely associated with physiological and pathological processes in animals (Egea et al., 2020). Low levels of ROS can act as a class of signalling molecules that regulate the basal cell activity; however when the rate of ROS accumulation in the body is higher than their elimination by the antioxidant system, the balance between oxidation and antioxidation in animals can be disrupted, predisposing them, e.g. to cellular oxidative stress, tissue damage and even the development of various diseases,

which in severe cases can be life-threatening (Yan et al., 2020). Of all the aforementioned negative processes, oxidative stress is the most likely to occur. As a component of the innate immune system, it plays a defensive role against invading pathogens. However, as a result of immune system mobilisation, the produced active substances can cause damage to somatic cells (Mirończuk-Chodakowska et al., 2018). *In vivo*, enzymatic and non-enzymatic antioxidants together form a complete antioxidant system (Poljsak et al., 2013; Lu et al., 2020), which maintains ROS levels in a stable but dynamic balance. Antioxidant enzymes produced by the body are the main components of the antioxidant system and these include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), etc. (Pederzolli et al., 2007). SOD is considered the oldest antioxidant enzyme in nature and it is the only enzyme in living organisms that can scavenge O_2^- (Frank et al., 1980). High concentrations of superoxide anion free radicals cause oxidative cell damage. SOD, as a type of metalloenzyme, contains metalloids as cofactors in the active site and reduces O_2^- by breaking down superoxide anion radicals into hydrogen peroxide and molecular oxygen through disproportionation reactions (Younus, 2018). Specifically, the catalytic role of SOD is the conversion of oxidized and reduced states through electron exchange with metal ions. Oxidised metal ions are reduced by superoxide anion radicals to generate oxygen, while reduced metal ions react with HO_2^- during re-oxidisation to produce hydrogen peroxide. Finally, hydrogen peroxide is catalysed by the enzyme catalase into water and oxygen.

Currently, in the context of intensive and large-scale broiler farming worldwide (Mottet and Tempio, 2017), when good rearing conditions are not provided, broilers suffer from chronic oxidative stress due to high temperature, excessive breeding density and other causes, which can even lead to high disease and death incidence due to reduced immunity (Simitzis et al., 2012). The reason for this is that under high temperature conditions, the amount of oxygen free radicals in the body increases, resulting in an increase in lipid peroxides, protein oxidation products and DNA oxidation damage products. Accumulation of these compounds impairs immune function and ultimately affects production performance. Antioxidants represented by vitamin E and polyphenols (Yan et al., 2020) are widely used in livestock and poultry production. However, SOD and common antioxidants have different chemical structures and their physiological functions are not consistent. Therefore, it is necessary to investigate whether SOD exerts a better effect on animals than other antioxidants. SOD has been extensively studied in medicine, mainly for its antioxidant, anti-inflammatory, anti-cancer and skin damage repair properties (Nozik-Grayck et al., 2005; Lund et al., 2009; Wu et al., 2012). However, its application as an antioxidant in broiler breeding has rarely been reported. In this context, the present study evaluated the effects of dietary SOD on growth performance, antioxidant capacity, digestive enzyme activity and serum biochemical indices in broilers.

Material and methods

All experimental procedures in this study were approved by the Hunan Agricultural University (File code: No. 2013-06).

The authors confirm that they have complied with the ethical policies of the journal as described in the guidelines for authors on the journal website, and have obtained approval from the appropriate ethical review board. The authors confirm that they adhered to EU standards for the protection of animals used for scientific purposes (and feed legislation, if applicable).

SOD source

SOD (enzyme activity was 20 000 U/g, powder) used in this study was provided by Shandong Long Kote Enzyme Preparation Co., Ltd (Linyi, SD, China). *Pichia pastoris* with manganese as a prosthetic group is fermented to produce SOD, and the enzyme liquid is subjected to ultra-fine filtration and subsequently spray-dried to produce a powdered SOD sample with ≥ 99% purity.

Animals and experimental treatments

A total of 320 one-day-old yellow-feather broilers (all test animals were male) were selected. The experimental period lasted from day 1 to day 28 after hatching. After 3 days of pre-feeding, the chicks were weighed. Their average 3-day initial body weight (IBW) was 74.00 ± 1.58 g. Yellow-feather broilers were randomly allocated to 5 treatment groups with 8 replicates, 8 broilers each. Each replicate was housed in a separate cage (120 cm long × 80 cm wide × 50 cm high) under natural environmental conditions and equipped with nipple drinkers and feeders. The control group of broilers was fed the basal diet, while 4 experimental group were supplemented with 100 mg/kg (ppm), 200 mg/kg, 400 mg/kg and 800 mg/kg SOD, respectively. The basal diet was

formulated according to the Nutritional requirements for yellow-feather broilers (NY/T 3645-2020) and the composition and nutrient contents of the feeds are shown in Table 1. Feed supplementation was carried out three times a day at 8 am, 1 pm and 6 pm to ensure free feed intake by broilers and adequate, hygienic drinking water. The external temperature during breeding was high, averaging 35 ± 2 °C. The light cycle was 20 h of light and 4 h of darkness. Individuals in each replicate were distinguished by foot rings to facilitate subsequent testing. Initial weights, final weights and daily feed intake were recorded for each replicate, and the average daily gain (ADG), average daily feed intake (ADFI) and feed-to-gain ratio (F:G) were determined.

Table 1. Basal experimental diet composition and nutrient levels (on air-dried basis, %)

Items	Ingredient, %	Items	Nutrient levels[2], %
Corn	58.00	Metabolic energy, MJ/kg	12.54
Soybean meal	33.13	Crude protein	20.93
Fish meal	1.70	Lysine	1.11
Soybean oil	3.20	Methionine	0.50
DL-methionine	0.17	Methionine + cystine	1.84
$CaHPO_4$	1.50	Calcium	1.00
Limestone	1.00	Available phosphorus	0.47
Salt	0.30		
Premix[1]	1.00		

[1] premix, provided per kg of complete diet: IU: vit. A 12 000, vit. D_3 3 000, vit. E 25; mg: vit. K_3 2.6, vit. B_1 2.3, vit. B_2 8, vit. B_3 (niacin) 35, vit. B_5 (pantothenic acid) 12, vit. B_6 4, vit. B_7 (biotin) 0.18, vit B_9 (folic acid) 0.6, vit. B_{12} 0.015, copper 8, iron 100, manganese 120, zinc 100, iodine 1, selenium 0.3; [2] total energy and crude protein content were measured; calcium, available phosphorus and amino acid composition were analysed

Sample collection

At the end of the trial, the remaining feed in the through was cleaned. Eight chickens from different treatment groups were selected and fasted for 12 h before slaughter. Prior to slaughter, 30 ml of venous blood were collected. Whole blood was left at room temperature for 30 min and then centrifuged at 3 500 rpm, 4 °C for 10 min to separate the serum. It was aliquoted into 1.5-ml EP tubes and quickly transferred to 80 °C freezer and stored until analysis. The major organs (heart, liver, spleen, muscular stomach, pancreas and bursa of Fabricius) were weighed and the lengths of individual intestinal segments and pH of their contents were measured. Subsequently, the liver and intestinal mucosa (duodenum, jejunum, ileum) were collected as required. Individual intestinal segments were rinsed with saline solution and the intestinal walls were broken open to collect the mucosa and rapidly frozen in liquid nitrogen and transferred to −80 °C after complete freezing.

Organ index

After slaughter, the heart, liver, spleen, gizzard, pancreas, and bursa were divided and weighed separately. Calculations were made according to the following formula: relative organ weight = (fresh organ weight / live weight) × 100%.

Intestinal length, weight and content pH

The post-mortem broiler intestine was divided into three parts: duodenum, jejunum, and ileum. The lengths of the intestinal sections were measured with a ruler, the weights were determined using an analytical balance and the pH of the intestinal contents was measured with a pH meter (PHS-2F, INESA Scientific Instrument Co., Ltd, Shanghai, SH, China).

Antioxidant parameters

The indices tested included total antioxidant capacity (T-AOC), SOD, CAT and GSH-Px activities and malondiadehyde (MDA) content, and were analysed calorimetrically using an infinite F50 spectrophotometer (Tecan Trading AG, Männedorf, Switzerland); the test samples were serum and liver. Commercial kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, JS, China) and used according to the instructions.

Before the experiment, the serum was thawed on ice and centrifuged again before use. The liver was removed from −80 °C and the appropriate amount of sample was divided and homogenized according to the instructions. After centrifugation, the supernatant was used for assays.

Catalogue numbers of the kits used: T-AOC, A015-2-1; SOD, A001-3-2; CAT, A007-1-1; GSH-Px, A005-1-2; MDA, A003-1-2.

Digestive enzyme activity

Trypsin, α-amylase and lipase levels were measured in the duodenum, jejunum and ileum. Mucosa from individual intestinal segments was homogenized with a JXFSTPRP-24 grinder (Shanghai Jingxin Industrial Development Co., Ltd, Shanghai, SH, China), assayed according to the instructions and analysed colorimetrically using an infinite F50 spectrophotometer (Tecan Trading AG, Männedorf, Switzerland). Commercial kits were purchased from

Beijing Soleibao Technology Co., Ltd (Beijing, BJ, China). Catalogue numbers of the kits used: trypsin, BC2310; α-amylase, BC0615; lipase, BC2340.

Serum biochemical indices

Serum enzyme activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) were determined. Serum concentrations of triglycerides (TG), total cholesterol (CHOL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were analysed using a Mindray fully automatic biochemical analyser BS-350E (Shenzhen Mindray Biological medical Electronics Co., Ltd, Shenzhen, GD, China) and commercial kits (Shenzhen Mindray Biological Medical Electronics Co., Ltd, Shenzhen, GD, China) according to the company's instruction books.

Statistical analysis

Data obtained from all experiments were summarized using Excel software (Microsoft Co., Redmond, WA, USA), entered into SPSS 20 software (International Business Machines Co., Armonk, NY, USA) and subsequently analysed using one-way ANOVA. Duncan's multiple comparison test was used to test the homogeneity of variance. The results were expressed as arithmetic means and SEM, and a difference was considered statistically significant when the *P*-value was < 0.05.

Results

Growth performance

Table 2 shows that dietary SOD supplementation significantly increased FBW and ADG in broilers ($P < 0.05$), while significantly reduced F:G ($P < 0.05$) without affecting ADFI ($P > 0.05$). The growth promoting effect of broilers became more effective with increasing SOD supplementation level.

Table 2. Effects of dietary superoxide dismutase (SOD) supplementation on growth performance of broilers

Item	SOD inclusion level, mg/kg					SEM	*P*-value
	0	100	200	400	800		
IBW, g	74.00	74.17	73.83	73.83	74.17	0.56	0.95
FBW, g	693.83^{b}	720.17^{ab}	745.17^{a}	722.00^{ab}	748.00^{a}	15.81	0.01
ADFI, g/d	40.52	38.26	38.23	37.71	31.73	3.61	0.19
ADG, g/d	22.14^{c}	23.07^{b}	23.99^{a}	23.16^{b}	24.06^{a}	0.23	<0.01
F:G	1.84^{a}	1.66^{b}	1.60^{b}	1.63^{b}	1.54^{b}	0.06	<0.01

IBW – initial body weight, FBW – final body weight, ADFI – average daily feed intake, ADG – average daily gain, F:G – feed:gain ratio, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Organ indices

The results for organ indices are shown in Table 3. The addition of different SOD levels improved the cardiac index ($P < 0.05$), and the addition of 200 g/T improved the liver and spleen indices ($P < 0.05$). At the same time, the liver, spleen and gizzard indices decreased significantly ($P < 0.05$) with increasing supplementation, especially at 800 mg/kg. The results also showed that SOD addition exerted a lowering effect on the bursa of Fabricius index ($P < 0.05$).

Table 3. Effects of dietary superoxide dismutase (SOD) supplementation on organ indices of broilers, %

Item[1]	SOD inclusion level, mg/kg					SEM	*P*-value
	0	100	200	400	800		
Cardiac index	0.41^{b}	0.50^{ab}	0.52^{a}	0.57^{a}	0.58^{a}	0.05	0.02
Liver index	2.14^{b}	2.02^{b}	2.64^{a}	2.05^{b}	1.90^{b}	0.13	<0.01
Spleen index	0.16^{b}	0.17^{b}	0.22^{a}	0.15^{b}	0.16^{b}	0.02	0.02
Gizzard index	1.70^{bc}	1.94^{a}	1.53^{c}	1.82^{ab}	1.52^{c}	0.10	<0.01
Pancreas index	0.29	0.32	0.28	0.27	0.27	0.02	0.06
Bursa of Fabricius index	0.34^{a}	0.29^{bc}	0.25^{c}	0.31^{ab}	0.25^{c}	0.02	<0.01

[1] organ indices = (organ weight/carcass weight) × 100%; SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Intestinal indices

The results for broiler intestinal length, weight and pH of intestinal contents are shown in Table 4. The SOD intervention did not affect the length and weight ($P > 0.05$) of the duodenum, jejunum and ileum of broilers, but significantly decreased the pH in the jejunum ($P < 0.05$).

Table 4. Effects of dietary superoxide dismutase (SOD) supplementation on intestinal indices of broilers

Item	SOD inclusion level, mg/kg					SEM	*P*-value
	0	100	200	400	800		
Length, cm							
duodenum	19.2	20.5	19.3	20.3	19.5	1.08	0.26
jejunum	37.5	37.1	38.65	38.75	36.65	1.36	0.45
ileum	26.82	26.46	27.65	24.65	24.65	1.58	0.25
Weight, g							
duodenum	7.27	6.72	6.87	6.90	6.67	0.50	0.78
jejunum	10.03	11.19	10.78	10.56	11.05	0.57	0.30
ileum	6.67	6.73	6.27	6.42	6.77	0.59	0.89
pH							
duodenum	6.42	6.40	6.57	6.62	6.47	0.11	0.18
jejunum	6.48^{a}	6.25^{b}	6.34^{ab}	6.27^{b}	6.23^{b}	0.07	0.02
ileum	6.76	6.88	6.61	6.91	6.81	0.14	0.28

SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Antioxidant parameters

The results of serum and liver antioxidant indices are shown in Table 5. The addition of different SOD levels to the diet significantly increased serum T-AOC and GSH-Px ($P < 0.05$), and significantly reduced MDA content ($P < 0.05$), but did not affect the activity of T-SOD and CAT ($P > 0.05$). However, the activity of T-SOD, CAT, and GSH-Px in the liver was significantly increased ($P < 0.05$), while T-AOC and MDA were not affected ($P > 0.05$).

Digestive enzyme activities

Diet supplementation with different levels of SOD did not alter the activity of amylase, trypsin and lipase in the duodenum, jejunum, and ileum ($P > 0.05$) (Table 6), which indicated that dietary nutrient digestion in broilers was not affected.

Serum biochemical indices

The addition of SOD at doses ranging from 100 mg/kg to 800 mg/kg caused a decrease, subsequent increase in ALT activity ($P < 0.05$) and highly significant elevation of AST activity ($P < 0.05$). In addition, no marked differences were recorded for ALP, TG, CHOL, HDL, and LDL indices ($P > 0.05$) (Table 7).

Table 5. Effects of dietary superoxide dismutase (SOD) supplementation on antioxidant parameters of broilers

Item	SOD inclusion level, mg/kg					SEM	*P*-value
	0	100	200	400	800		
Blood serum, U/ml							
T-AOC	5.26^{b}	7.18^{a}	7.03^{a}	7.98^{a}	7.72^{a}	0.61	<0.01
T-SOD	170.69	172.43	174.55	175.29	167.86	4.58	0.50
CAT	4.39	4.98	5.01	4.50	5.06	0.29	0.07
GSH-Px	1423.93^{b}	1412.5^{b}	1515.17^{ab}	1555.52^{a}	1562.28^{a}	57.08	0.03
MDA, nmol/ml	7.21^{a}	6.12^{ab}	6.29^{ab}	5.49^{b}	4.98^{b}	0.70	0.04
Liver, U/mg prot.							
T-AOC	6.17	6.59	7.12	6.52	6.46	0.40	0.22
T-SOD	67.60^{b}	104.16^{a}	89.69^{a}	99.94^{a}	98.40^{a}	6.76	<0.01
CAT	5.73^{c}	8.02^{ab}	8.38^{a}	7.07^{b}	7.14^{b}	0.53	<0.01
GSH-Px	402.70^{c}	441.31^{bc}	586.20^{ab}	707.48^{a}	670.07^{a}	75.81	<0.01
MDA, nmol/mg prot.	1.76	1.60	2.37	1.50	1.92	0.30	0.07

T-AOC – total antioxidant capacity, T-SOD – total superoxide dismutase, CAT – catalase, GSH-Px – glutathione peroxidase, MDA – malondialdehyde, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 6. Effects of dietary superoxide dismutase (SOD) supplementation on digestive enzyme activities of broilers (U/mg prot.)

Item	SOD inclusion level, mg/kg					SEM	*P*-value
	0	100	200	400	800		
α-Amylase							
duodenum	1.60	1.94	1.75	1.81	1.96	0.20	0.40
jejunum	2.66	2.55	2.70	2.90	2.82	0.18	0.38
ileum	1.65	1.66	1.60	1.53	1.79	0.16	0.60
Trypsin							
duodenum	681.01	682.95	669.74	672.24	698.02	33.25	0.92
jejunum	798.45	880.31	874.76	879.52	869.50	42.82	0.29
ileum	709.94	682.95	691.14	697.99	698.02	31.47	0.94
Lipase							
duodenum	3.65	3.69	3.91	3.83	4.03	0.45	0.91
jejunum	3.50	3.33	3.43	3.47	3.76	0.35	0.79
ileum	3.19	3.43	3.45	3.51	3.21	0.41	0.90

SEM – standard error of the mean; $P > 0.05$

Table 7. Effects of dietary superoxide dismutase (SOD) supplementation on serum biochemical indices of broilers

Item	SOD inclusion level, mg/kg					SEM	*P*-value
	0	100	200	400	800		
ALT, U/l	3.74^{b}	2.94^{c}	3.64^{bc}	4.11^{b}	6.14^{a}	0.35	<0.01
AST, U/l	259.09^{a}	202.11^{c}	234.41^{b}	191.68^{c}	203.09^{c}	7.27	<0.01
ALP, U/l	2594.20	2763.95	2479.87	2396.05	2467.79	307.99	0.78
TG, mmol/l	0.30	0.32	0.28	0.31	0.30	0.02	0.16
CHOL, mmol/l	3.47	3.25	3.03	3.32	3.36	0.16	0.10
HDL, mmol/l	0.63	0.55	0.61	0.66	0.52	0.09	0.53
LDL, mmol/l	2.27	2.33	1.96	1.95	2.21	0.23	0.33

ALT – alanine transaminase, AST – aspartate transaminase, ALP – alkaline phosphatase, TG – triglycerides, CHOL – total cholesterol, HDL – high-density lipoprotein, LDL – low-density lipoprotein, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Discussion

SOD belongs to a class of antioxidant enzymes with specific functions and is also known as the first line of defence for organisms in terms of its antioxidant functions. It converts O_2^- into hydrogen peroxide (H_2O_2) and oxygen (O_2) through a disproportionation reaction (Azab et al., 2019), and subsequently H_2O_2 is used by CAT and converted into completely harmless water (H_2O) (Ho et al., 2004). Around 1990, SOD was first introduced to the market. After nearly 30 years of continuous research and development of the enzyme industry, SOD now has a variety of applications, including in food and drug processing, cosmetics, medical therapies, as well as in animal husbandry (Bafana et al., 2011). This study investigated the effects of dietary SOD supplementation on growth performance, antioxidant capacity and digestive enzyme activities in yellow-feather broilers under summer environmental conditions. The present work provides the basis for the application of SOD in broiler breeding.

Effects of SOD supplementation on growth performance. The application of antioxidants can improve animal performance, which is supported by a large number of scientific studies (Miller et al., 1993; Chauhan et al., 2014; Shakeri et al., 2019). Similar results were also obtained in the present experiment. SOD did not influence the palatability of the diet, thus ADFI was not affected. However, under this assumption, both FBW and ADG improved significantly, while the F:G ratio decreased. SOD has been shown to improve the performance of broiler chickens, and the growth-promoting effect gradually increased with incremental SOD doses. This experiment was carried out during high temperatures of the external environment, which can induce oxidative stress in broiler chicken that is difficult to directly observe. Therefore, we believe that SOD could alleviate oxidative stress, thereby improving growth performance. Some researchers used SOD-rich melon pulp concentrate in pigs subjected to oxidative stress and obtained similar results in terms of growth performance (Ahasan et al., 2018).

Effects of SOD supplementation on organ development. Normal development of animal organs is an important basis for vital functions, and the degree of organ development is also a reference factor in assessing animal growth (Black et al., 1989). The organ index is often applied to characterise the status of animal organ development. In this study, the group receiving 200 mg/kg SOD improved the liver and spleen indices of broilers, and SOD intervention significantly reduced the weight of the bursa of Fabricius. It appears that the liver, spleen, and bursa of Fabricius are organs that play immune functions in broilers (Zhang et al., 2021), and the reasons for this result require further research. Interestingly, compared to the control group, chickens receiving SOD had an increased heart weight, which was consistent with the result of previous study using coenzyme Q_{10} (antioxidant) (Geng et al., 2007). Hence, the question is whether different antioxidants have the ability to increase the mass of an animal's heart, and what are the mechanisms involved in this process?

Assessing animal's health based on the intestinal structure and functional integrity is now an established method (Celi et al., 2019). As the largest organ in the animal's body, the gut, in addition to digestion and absorption of nutrients, performs a variety of biological functions, e.g. related to immunity and excretion (Qi et al., 2020). We found that SOD did not affect intestinal function and the weight of individual intestinal segments in broilers, but it did significantly lower the jejunum pH. The pH of the digestive tract gradually increases from the duodenum through jejunum to the ileum. Its value is mainly determined by the secretion of hydrochloric acid,

bile, and pancreatic juice (Mazukhina et al., 2020; Rønnestad and Morais, 2020). An acidic environment can not only facilitate the breakdown and full absorption of dietary nutrients, but also promote the growth of beneficial bacteria and inhibit pathogenic microorganisms, thereby improving the body's disease resistance and dietary utilisation rates (Ratzke and Gore, 2018; Pearlin et al., 2020).

Effects of SOD supplementation on antioxidant parameters. As we all know, the antioxidant system of the animal body is composed of enzymes and non-enzymatic antioxidant components, and SOD, CAT and GSH-Px are the main enzymes that form the antioxidant system (Ma et al., 2017). These enzymes interrupt the oxidation reaction in the cell and free radicals lose their activity before attacking the cell. The mechanism generally involves several aspects. The first is to reduce the energy state of free radicals, and subsequently neutralize free radical electrons to stabilize them (Krishnamurthy and Wadhwani, 2012). Increasing the content of antioxidant enzymes can improve the ability of broilers to withstand oxidative stress, promote growth and improve disease resistance (Surai et al., 2019a; b). In this study, SOD supplementation helped to improve the animals' antioxidant capacity, which was manifested in both blood and liver parameters. The improvement of antioxidant capacity may be an important factor promoting the growth of broilers, especially in an environment with high summer temperatures. The use of SOD as an additive (antioxidant) has rarely been applied in animal experiments on broiler chickens, but similar results were obtained for other antioxidants such as vitamin E (Yesilbag et al., 2011) or vitamin C + folic acid (Gouda et al., 2020).

As the largest detoxification organ in animals, the liver performs physiological functions. Why does SOD also have a growth-promoting effect while improving the antioxidant capacity of the liver? Some studies demonstrated that SOD inhibited the activity of *Escherichia coli, Staphylococcus aureus*, and *Micrococcus luteus*, thereby triggering an immune response and improving animal health. This could be an important reason for the increased growth performance of broilers (Zhang et al., 2007; González-Ruiz et al., 2021).

Effects of SOD supplementation on digestive enzyme activities. An important function of digestive enzymes in the small intestine is to decompose food that enters the digestive tract, so that nutrients can be readily absorbed and utilised by animals (Whitcomb and Lowe, 2007). Studies by other authors have found that an increase in digestive enzyme activity in the intestine helps to promote animal growth, but there are many factors that affect the activity of these enzymes, including diet composition, additives, microorganisms and others (Cao et al., 2020). In this study, the content of protease, amylase and lipase in each segment of the small intestine did not change, indicating that SOD did not affect the secretion of digestive enzymes.

Effects of SOD supplementation on serum biochemical indices. ALT and AST are important indicators reflecting liver function. ALT is secreted from damaged liver cells and is also the basis for assessing overall liver damage (Marchesini et al., 2008; Le Couteur et al., 2010). Data analysis demonstrated that as the level of SOD supplementation gradually increased, ALT activity tended to decline first and subsequently increase, indicating that a low SOD supplementation level could reduce the liver's metabolic load. However, when the dose reached 800 mg/kg, it could cause liver cell damage and increased ALT activity. In addition, different SOD supplementation levels significantly reduced AST activity – a transaminase with important physiological functions mainly present in organs and tissues such as myocardium and liver (Ndrepepa, 2021). When liver cells are damaged, AST enters the blood due to increased permeability of liver cells and is an auxiliary indicator of heart or liver disease (Sîrbu et al., 2016). The decrease in AST content in this experiment indicated that SOD addition improved the health status of the animals' liver. Dietary SOD supplementation had no effect on ALP, TG, CHOL, HDL and LDL. ALP is found mainly in the liver and bones and is an important metabolic regulatory enzyme. Similarly to TG, CHOL, HDL and LDL activity, ALP is closely associated with lipid metabolism (Pekarthy et al., 1972). The above results indicate that SOD does not affect the lipid metabolism of broilers.

Conclusions

Based on the obtained data, it can be concluded that SOD improved the early growth performance and antioxidant capacity of yellow-feather broilers. These data support the application of SOD in animal husbandry, as well as help to promote this new feed additive in the livestock industry. Based on the above results, we believe that SOD in yellow feather broilers should be supplemented at a dose of 400–800 mg/kg.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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